Anti-Inflammatory Effects of Ergotamine in Steers (44562)

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Abstract. **The objective of this experiment was to investigate whether the ergot alkaloid, ergotamine (ET), an alkaloid used to model fescue toxicosis in cattle, modifies the response of cattle to endotoxin (LPS) challenge. Steers (*n* = 16) were divided into the following treatment groups: control (C), ergotamine (ET), endotoxin (LPS), and ET + LPS. ET and ET + LPS groups received a single bolus intravenous injection of ET (40 μg · kg · body wt^{-1}), whereas C and LPS steers received a single bolus injection of sterile vehicle. Thirty minutes after ET/vehicle administration, a single bolus intravenous injection of LPS (0.2 μg · kg · body wt^{-1}) was given. Blood was collected at various time points for 48 hr post. Endotoxin increased rectal temperature (RT) and the circulating levels of tumor necrosis factor-α (TNF-α), cortisol, haptoglobin (Hp), thromboxane B_2 (TXB_2). The circulating Hp, TNF-α, and TXB_2 increases were blunted by pretreatment with ET compared with ET + LPS. Ergotamine by itself increased circulating cortisol and RT, whereas it decreased serum prolactin (PRL). Therefore, whereas administration of LPS at 0.2 μg/kg to steers resulted in an expected response, the combination of ET + LPS attenuated major effects of LPS alone. Thus, acute administration of ET appeared to be anti-inflammatory as it decreased the inflammatory response to LPS, an effect likely driven at least in part by the ET-caused cortisol increase.**

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An immune challenge (bacterial or viral infection, acute stress, or other nonpathogenic challenge) in food animals results in metabolic and endocrine shifts with decreased growth as an end result (1, 2). During an immune challenge, there are increased circulating inflammatory cytokines (interleukin-1 [IL-1], IL-6, and tumor necrosis factor-α [TNF-α]) and other immune modulators such as glucocorticoids and eicosanoids (3). Endotoxin (lipopolysaccharide, LPS) potently induces the inflammatory cytokines, cortisol, and the prostaglandin thromboxane-B_2 (TXB_2) in many species (3), including cattle (4). These in turn cause the acute phase response. In cattle, haptoglobin (Hp) is a major acute phase response protein synthesized in the liver (5, 6). Increasing amounts of this protein have been observed during an inflammatory process (6).

Tall fescue (*Festuca arundinacea* Schreb) is the predominant cool season forage in the piedmont and intermountain district of the South-east portion of the United States, but it is also grown in the northwest and the northeast parts of the country. However, most of the tall fescue is infected with an endophyte (*Neotyphodium coenophialum*) (7) that results in the elaboration of ergot alkaloids into the forage that are considered causative for fescue toxicosis in cattle, a condition associated with decreased growth and reproduction (8). The major types of ergot alkaloids (EA) produced by the endophyte are the clavine and the more biologically potent ergopeptide alkaloids (9). We recently reported that steers grazing endophyte-infected tall fescue for the duration of the grazing period and challenged with

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LPS had greater inflammatory response to LPS in terms of increased circulating TNF-α and cortisol levels, and decreased insulin-like growth factor-1 (IGF-1) compared with steers grazing endophyte-free tall fescue (10). The objective of this experiment was to determine if acute administration of ergotamine (ET), an ergopeptide alkaloid as that found in endophyte-infected tall fescue (9) to steers, would also enhance the inflammatory response to LPS.

Materials and Methods

Animals. The experiment was conducted in the spring of 1998 (April 22–24), and the mean ambient temperature was 14°C. Angus yearling steers ($n = 16$) were maintained on cereal rye (S*ecale cereale* L). Steers were withheld from water for 16 hr and weighed (201.9 kg ± 10.5, mean ± SD) on the day before the experiment. Animals were ranked by weight from lowest to highest and assigned to groups of four on the basis of rank. Then, a steer from each group was randomly assigned to one of four groups ($n = 4$/group). Following group assignment, each group was randomly assigned to a treatment ($n = 4$/treatment). The treatments were as follows: control (C), ergotamine (ET), endotoxin (lipopolysaccharide, LPS), and ergotamine plus endotoxin (ET + LPS). Following weighing and assignment to treatment, an indwelling cannula (Tygon, 1.02 mm i.d., 1.78 mm o.d.) was placed in the jugular vein for administration of LPS/ET/saline and blood collection. On the day of the experiment, animals were tethered in individual stalls and provided water *ad libitum*.

Treatment. Ergotamine tartrate (RBI, Natick, MA) was dissolved in a 20% solution (w/v) of 2-hydroxyprolyl-B-cyclodextrin (RBI, Natick, MA) in sterile double-distilled (dd) water immediately prior to administration and protected from light. Endotoxin (*Escherichia coli; 055:B5*, Sigma, St. Louis, MO) was reconstituted with sterile 0.1% BSA phosphate-buffered saline (PBS) solution to a stock concentration of 0.1 mg/ml. Immediately before administration, stock LPS solution was further diluted with PBS to 8 μg LPS/ml concentration.

At time −30 min, ET and ET + LPS treatments received a single bolus intravenous injection of ET (40 μg·kg·body wt^{-1}, ≈ 2.7ml vehicle volume), whereas C and LPS steers received a bolus injection of sterile vehicle (2.7ml). Thirty minutes after ET/vehicle administration (Time 0), a single bolus intravenous injection of LPS (0.2 μg·kg·body wt^{1}, ≈ 5 ml vehicle volume) was administered to LPS and ET + LPS treatments whereas C and ET treatments received 5 ml of sterile PBS. Following administration, articles were flushed in with 10 ml sterile 3.5% sodium citrate solution.

Data Collection. Before administration of ET/vehicle, two sham blood samples (10 ml/sample) were collected 30 min apart to accustom animals to blood collection. Then, a blood sample was collected at time −30 min for determination of basal levels. At Time 0, another blood sample was taken, immediately followed by LPS/PBS administration. After LPS/PBS administration, samples were taken at 30 min, 1, 2, 3, 4, 5, 6, 12, 24, and 48 hr. Steers were not fed for the first 12 hr, then returned to pasture and brought back to sampling stalls for the additional blood collections at 24 and 48 hr. Blood (20 ml) was collected into tubes with EDTA for plasma and without additives for serum harvesting (Vacutainer, Becton Dickinson, Rutherford, NJ) and kept on ice until harvest. Following harvest, plasma/serum was aliquotted and stored at −70°C until assayed. In addition to blood collection, rectal temperature was measured hourly, beginning at time −30 min, using a digital thermometer (B-D, Becton Dickinson, Franklin Lakes, NJ).

Assay Procedures. *Prolactin (PRL).* Serum concentrations of PRL were determined by radioimmunoassay (RIA) procedure (11) with reagents supplied by the USDA Hormone Distribution Program (Beltsville, MD). The intra- and interassay coefficients of variation were 3.7% and 7.3%, respectively.

Cortisol. Serum cortisol was determined with a commercially available solid phase RIA kit (Diagnostic Products Corp., Los Angeles, CA). All samples were assayed together. The intra-assay CV was 7.9%.

Tumor necrosis factor-α (TNF-α). Plasma immunoreactive TNF-α was measured by a specific RIA for cattle (12). All samples were assayed together. The intra-assay CV was 9.2%.

Haptoglobin (Hp). Serum concentration of Hp was determined by an ELISA according to Young *et al*. (13) using plates coated with hemoglobin for capture and antihaptoglobin monoclonal antibody for detection of serum Hp.

Thromboxane-B_2 (TXB_2). Plasma concentrations of TXB_2 were measured by a commercially available enzyme immunoassay (EIA, Amersham Life Sciences Inc., Arlington Heights, IL). Before assaying, TXB_2 was extracted from the plasma using a solid-phase extraction procedure recommended by the manufacturer. Briefly, 1ml of plasma was acidified to pH 3.0 with 1*M* citric acid (≈ 0.25 ml). Amprep C2 columns (Amersham Life Sciences Inc., Arlington Heights, IL) were conditioned by rinsing with 2 ml of methanol followed by 2 ml of water rinse. Then, the acidified plasma samples were applied to the columns. The columns were washed with 5 ml of water, followed by 5 ml of 10% ethanol, and 5 ml of hexane. Thromboxane B_2 was eluted with 5 ml of methyl formate. The methyl formate was vacuum evaporated, and the extract reconstituted in the EIA buffer. A known amount (100 pg) of TXB_2 was added to selective tubes to monitor recovery. Mean recovery was 91%.

Statistical Analysis. Analysis was performed using the Statistix Analytical Software (Tallahassee, FL). Data are expressed as mean ± SEM. Data were analyzed by a 2 × 2 factorial model split-plot-in-time with ET (ET versus vehicle), LPS (LPS versus vehicle), and their interaction as main factors and time as the subplot using a two-way analysis of variance (ANOVA). The appropriate means of significant ($P < 0.05$) main effects or their interaction were

Table I. Serum Haptoglobin (Hp) (mean ± SEM, mg/dl) in Steers Administered Ergotamine, 40 µg·kg·body wt^{-1} or Vehicle at Time −30 min Followed by 0.2 µg·kg·body wt^{-1} iv Endotoxin (LPS) at Time 0

Treatments	Time				
	0	0–6 hr	12 hr	24 hr	48 hr
C	1.3 ± 0.16	1.3 ± 0.15	1.4 ± 0.23	1.2 ± 0.11	1.1 ± 0.06
ET	1.1 ± 0.10	1.1 ± 0.13	1.1 ± 0.08	1.1 ± 0.07	1.0 ± 0.00
ET + LPS	1.3 ± 0.23	1.4 ± 0.26	[a]4.4 ± 1.69*	[a]12.2 ± 4.84‡	[a]4.4 ± 0.61‡
LPS	1.1 ± 0.06	1.3 ± 0.07	[a]2.4 ± 0.27†	[a]9.7 ± 4.05‡	[b]26.0 ± 18.18‡

Note. C is control, ET is ergotamine, LPS is lipopolysaccharide (endotoxin). For statistical purposes, samples with Hp values of <1 mg/dl were assigned value of 1 mg/dl.
* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ vs Time 0 within treatment.
[a,b] Means with different superscripts are different from each other and from means without any letter designation at a specific time point ($P < 0.05$).

separated by Fisher's protected LSD *post hoc* test. A trapezoidal summation of individual areas (time on the *x*-axis and magnitude of the response on the *y*-axis) was used for calculation of responses over a time period where appropriate. Basal areas corresponding to the rectangle derived by multiplying *y*-value at Time 0 and total time were subtracted from total areas, and net value was the absolute area under the concentration curve (AUC) response. Because of this calculation approach, downward responses have negative AUC values, whereas upward responses have positive AUC values. The AUC data were analyzed by one-way ANOVA, followed by Fisher's protected LSD *post hoc* test.

Results

Serum Hp was unaffected by ET (Table I), but there was a main effect of LPS ($P < 0.01$) which was also time-dependent (LPS × time, $P < 0.01$). Both ET + LPS and LPS treatments had increased serum Hp at 12 hr, and Hp continued to rise until 24 hr (ET + LPS), or until the end of the sampling period (LPS). Serum Hp in both ET + LPS and LPS groups was greater ($P < 0.05$) compared with C and ET at 12, 24, and 48 hr. At 48 hr, serum Hp was increased the greatest ($P < 0.05$).

Highly significant interaction between ET and LPS influenced plasma TNF-α values (ET × LPS, ET × LPS × time, $P < 0.0001$; Fig. 1). Plasma TNF-α was unchanged in the C and ET groups. Compared with C plasma, TNF-α values were increased by ET + LPS (30 min–2 hr), and LPS (30 min–3 hr). Pretreatment with ET in the ET + LPS group reduced ($P < 0.001$) TNF-α compared with LPS at 30 min, 1, 2, and 3 hr. The area under the response curve (0–6 hr) was greatest for LPS (5.93 ± 0.38, mean ± SEM; AUC units) and ET + LPS was greater (1.71 ± 0.39 AUC units) compared with C and ET (0.11 ± 0.03 and −0.05 ± 0.04 AUC units, respectively; $P < 0.01$).

Serum cortisol increased following administration of either ET or LPS, but there was also a significant interaction between them (ET × LPS, $P < 0.01$; ET × LPS × time, $P < 0.05$; Fig. 2). Serum cortisol was abruptly increased ($P < 0.001$) in the ET (0–2 hr) and ET + LPS (0–4 hr) groups compared with pretreatment. Following LPS, serum cortisol was elevated ($P < 0.001$) from 1 to 6 hr compared with pretreatment. While both ET and LPS elevated serum cortisol, the effect of ET was temporally shorter. Maximal serum cortisol was 20% greater in the LPS versus ET + LPS. Cortisol values in the C slowly decreased over time and were significantly ($P < 0.05$) lower (2–12 hr) compared with pretreatment.

The increase of plasma TXB_2 in ET + LPS (1–3 hr), and LPS (1–4 hr) groups compared with controls (Fig. 3, top) was LPS-driven (LPS effect, $P < 0.0001$), but there was also a significant ET × LPS interaction (ET × LPS × time, $P < 0.001$). Pretreatment with ET in animals challenged with LPS blunted the increase in circulating TXB_2 during the first 4 hr following LPS, and at 1 and 4 hr post-LPS, this effect was significant (ET + LPS versus LPS, $P < 0.05$). In both ET + LPS and LPS, plasma TXB_2 returned to baseline at 4 and 6 hr, respectively. Similarly, TXB_2 area under the curve response (0–12 hr) for LPS (6279 ± 965.6, mean ± SEM, AUC units) was greatest ($P < 0.05$), whereas ET + LPS (3110 ± 286.7, AUC units) was greater ($P < 0.05$)

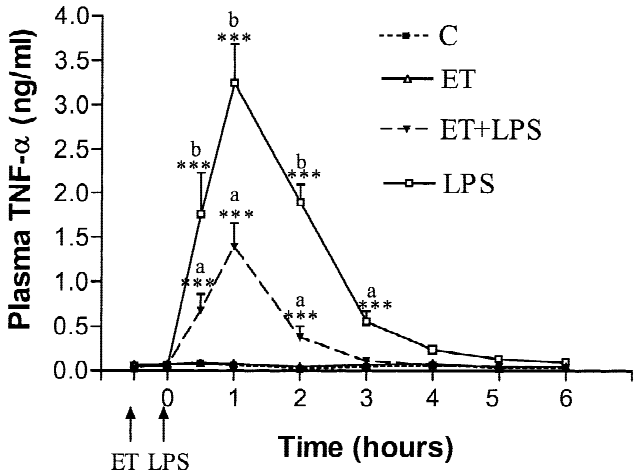

Figure 1. Plasma TNF-α (mean ± SEM) in steers ($n = 16$) given 40 µg·kg·body wt^{-1} ergotamine iv (ET, ET + LPS) or vehicle (C, LPS) at time −30 min followed by an endotoxin (LPS) challenge (0.2 µg·kg·body wt^{-1}) intravenously at Time 0 (LPS, ET + LPS). ***$P <$ 0.001 indicates differences within the same treatment compared with pretreatment values. [a,b]Means with different letters are different from each other and from means without any letter designation at a specific time point ($P < 0.05$). See Table I for treatment abbreviations.

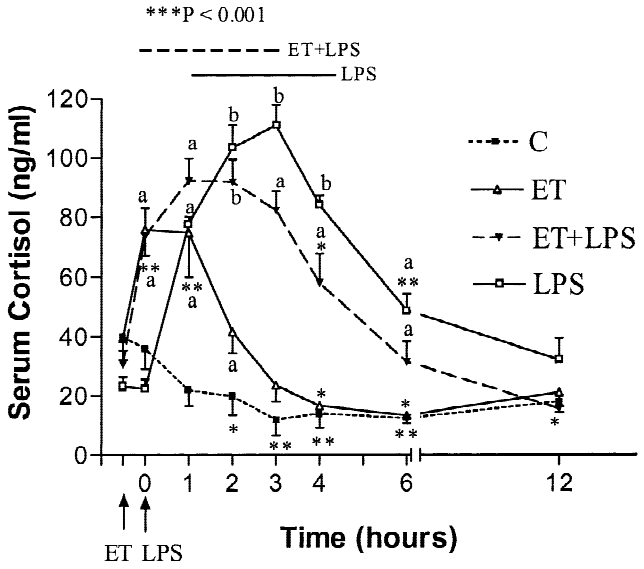

Figure 2. Serum cortisol (mean ± SEM) in steers given ergotamine followed by LPS. All values returned to baseline at 12 hr and did not differ thereafter (not shown). *P < 0.05, **P < 0.01, ***P < 0.001 indicate differences within the same treatment compared with pretreatment values. Length of lines indicates the duration of an effect within treatment (treatment designation marked at one end of the line), whereas the P-value above the lines indicates the minimal level of significance for the marked periods. [a,b]Means with different letters are different from each other and from means without any letter designation at a specific time point (P < 0.05). See Figure 1 for dosages and Table I for treatment abbreviations.

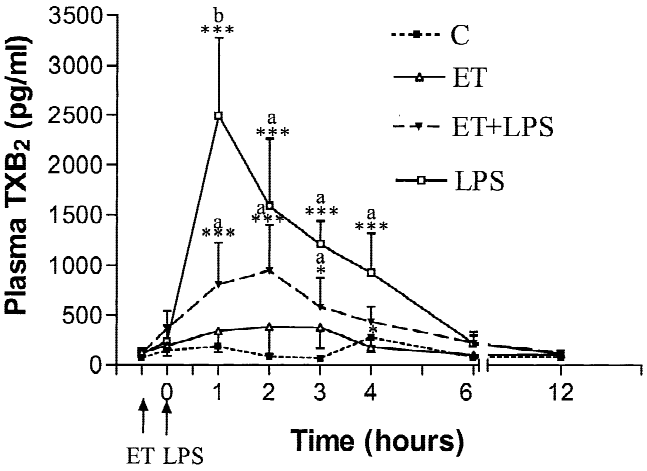

Figure 3. Plasma thromboxane B_2 (TXB_2, mean ± SEM) in steers given ergotamine followed by an LPS challenge. Values post-12 hr were not different (not shown *P < 0.05, ***P < 0.001 indicate differences within the same treatment compared with pretreatment values. [a,b]Means with different letters are different from each other and from means without any letter designation at a specific time point (P < 0.05). See Figure 1 for dosages and Table I for treatment abbreviations.

compared with C (536.9 ± 122.1, AUC units). ET alone failed to modulate TXB_2.

Similar to the cortisol response, rectal temperatures increased following administration of either ET or LPS, but there was also a significant interaction between them (ET × LPS × time, P < 0.05; Fig. 4). The maximal temperature

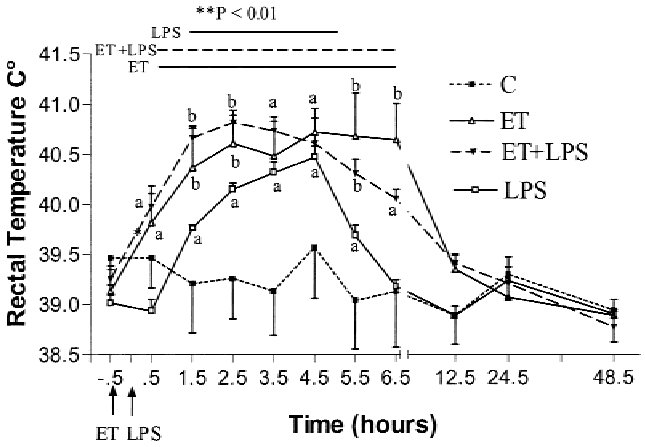

Figure 4. Rectal temperature (RT, mean ± SEM). **P < 0.01 indicates differences within the same treatment compared with pretreatment values. [a,b]Means with different letters are different from each other and from means without any letter designation at a specific time point (P < 0.05). See Figure 1 for dosages, Figure 2 for lines designation, and Table I for treatment abbreviations.

increment of the increase in the ET and ET + LPS groups was greater (P < 0.05) than the increase following LPS alone. Rectal temperature for ET and ET + LPS returned to baseline by 12.5 hr; however, rectal temperature following LPS returned to baseline more quickly (6.5 hr).

As a positive control, serum PRL decreased (maximum 81%) in the ET and ET + LPS groups from 30 min to 4 hr, an effect driven by ET (P < 0.001). LPS on the other hand, failed to alter serum PRL levels (P > 0.3) nor there was an ET × LPS interaction (P > 0.4). The overall basal PRL values were 6.2 ± 0.93 (mean ± SEM, ng/ml), and they decreased to 0.84 ± 0.14 and 1.15 ± 0.21 (mean 30 min–4 hr-values) in the ET and ET + LPS group, respectively. By 6 hr both ET and ET + LPS serum PRL values returned to normal. Since the decrement in serum PRL was similar in both ET and ET + LPS, this suggests that the decrease in serum PRL was a result of ET treatment.

Discussion

The findings of this investigation indicate that ET reduced the inflammatory response to LPS. The blunted increase in circulating Hp, TNF-α, and TXB_2 in the ET + LPS group compared with LPS alone supports this statement. At least part of the inflammatory response is mediated *via* cytokine production from monocytes, macrophages, and neural tissue (14). Following ET administration, there was an immediate increase in circulating cortisol similar to that reported by Browning *et al*. using a similar experimental paradigm (15). This effect of ET is attributed to both its adrenergic and serotonergic stimulating properties (16).

Dexamethasone treatment of macrophages from endotoxin-sensitive mice either before or simultaneously with LPS resulted in diminished LPS-induced secretion of TNF-α (17). Similarly, treatment of rats with dexamethasone at the time of LPS administration (18), or prior to LPS (19), reduced the LPS-induced TNF-α response. Therefore,

it is probable that the reduced TNF-α response to LPS in the steers given ET + LPS in the present experiment was the result of the prior increase in cortisol by ET. Not only can glucocorticoids directly inhibit TNF-α production and the subsequent inflammatory response, but also stress-increased corticosteroids are associated with increased systemic release of the anti-inflammatory cytokine IL-10 (20). It is recognized that LPS itself increased cortisol secretion in this experiment. A similar increase in adrenocortical activity following LPS was reported in cattle (4) as well as in both intact (21) and hypophysectomized (22) rats. Nevertheless, the prior increase in circulating cortisol in the steers as a result of ET may have resulted in not only the decreased TNF-α response but also the reduction of both TXB_2 and Hp. This is probable because increases in TNF-α ultimately result in a cascade effect that involves an increase in the inflammatory cytokines, induction of acute-phase proteins in the liver, and increased cyclooxygenase activity (23, 24).

Although ET-induced cortisol before LPS administration is considered a major factor for the observed anti-inflammatory effects of ET, other possible mechanisms are possible. Increased intracellular cyclic AMP levels have negatively affected TNF-α mRNA accumulation (25), and activation of D_1-like dopamine receptors increases intracellular cAMP (26). Ergotamine has the capacity to bind D_1 dopamine receptors (27) and bromocryptine, an ergot alkaloid similar to ET, has almost the same affinity for D_1 and D_5 dopamine receptors (28). Thereby, following activation of D_1-like receptors by ET, a mechanism is provided to decrease circulating TNF-α. In support of this mechanism, recent evidence indicates peripheral dopamine receptors in rat lymphocytes are predominantly D_5 type (29). In this regard, administration of a dopamine receptor agonist prior to induction of acute pancreatitis (severe inflammation) in rats reduced the onset and severity of the condition (30). Interestingly, increased intracellular cAMP levels are also associated with stimulation of the anti-inflammatory cytokine IL-10 in monocytes (20). Finally, suboptimal activation of the LPS receptor may also have occurred (31). Full activation of the LPS receptor and subsequent cytokines production and other inflammatory mediators is achieved only when the LPS receptor complexes with LPS associated with its binding protein (32). This LPS-binding protein is synthesized by hepatocytes, and its synthesis may have been decreased by acute administration of ET.

Activation of the pituitary D_2 receptors is inhibitory to prolactin secretion (33). The reduction in circulating PRL following ET in the steers indicated the effectiveness of treatment. Reduction of serum PRL in rats with a related ergot alkaloid, bromocryptine, was immunosuppressive following 1 week of treatment (34). Therefore, although it is possible that the reduced serum PRL in the steers could have affected the LPS-induced inflammatory response, it is not likely because of the brief decrement prior to LPS. The increase in RT following ET and LPS was expected. A body temperature increase following ET has been noted (35). This has been attributed to both vasoconstriction (36) and central effects (37). The hyperthermic effect of LPS is a central effect mediated partially by TNF-α (38).

Findings here of a decreased inflammatory response to LPS in the steers pretreated with ET differ dramatically from an earlier experiment in which an augmented inflammatory response to LPS occurred in steers that grazed ergot alkaloid-containing, endophyte-infected tall fescue (10). An explanation for these differences may be attributed to several factors. In the present experiment, ET was given acutely versus chronic exposure to ergot alkaloids over 8 months of grazing. Additionally, ET is a single compound compared with a diverse group of ergot and other alkaloids found in endophyte-infected tall fescue (8). The circulating concentration of ergot alkaloids at the time of LPS administration would undoubtedly differ in the two studies. Certainly, acute intravenous administration of ET as given here would be suspected to result in a greater circulating concentration of the administered agent at the time of LPS administration compared with that associated with grazing. Alternatively, the length of exposure rather than the circulating concentration of ergot alkaloids may be more important. Finally, and perhaps most importantly, circulating cortisol levels were not elevated prior to LPS with steers grazing endophyte-infected tall fescue (10).

A number of apparently disparate compounds have been shown to suppress LPS-induced TNF-α secretion. A variety of flavinoids with narginin being most potent in this regard suppressed LPS-induced TNF-α secretion (39). A substance from cinnamon bark when mixed with LPS inhibited the TNF-α–generating ability of LPS (40). Pretreatment of rats with pentoxifylline, a drug that inhibits cytokine production, inhibited LPS-induced TNF-α secretion (41), and adenosine attenuated the ability of monocytes to express TNF-α in response to LPS (42). Both adenosine and pentoxifylline (24) treatment resulted in an increased intracellular cAMP. Thus, the possibility that ET exerted its anti-inflammatory effects *via* D_5 receptor activation on immune cells and subsequent cAMP accumulation is pertinent. Moreover, combined application of dexamethasone and pentoxifylline to macrophages *in vitro* resulted in a greater suppression of TNF-α synthesis (*via* distinct mechanisms) than either agent alone (24). Therefore, acute ET administration may have used both cortisol-dependent and independent mechanisms for its anti-inflammatory effects.

In cattle, administration of recombinant bovine somatotropin diminished the LPS-induced release of TNF-α, cortisol, and TXB_2 (4). This effect may be caused by decreased binding of LPS to hepatic microsomal membranes. Several inducible hepatic enzymes such as alkaline phosphatase and aspartate aminotransferase are reduced in cattle grazing endophyte-infected tall fescue (43). Perhaps in the present experiment hepatic function was also reduced acutely following ET administration; therefore, there was a decreased TNF-α release from hepatic sources such as resident macrophages. This perhaps partially explains the decreased cir-

culating Hp following ET+LPS compared with LPS alone because this protein has a hepatic origin.

Thromboxane B_2 is the stable metabolite of thromboxane A_2 (TXA_2), a potent platelet aggregator and vasoconstrictor (44). The release in TXA_2 following LPS is considered causative for vascular shifts and pulmonary hypertension (45). The increase in circulating TXB_2 in response to LPS in cattle found here agrees with a previous report (4); however, the increase was blunted by prior administration of ET. Because TNF-α is a major stimulus for TXB_2, the decrease in TNF-α secretion following ET + LPS compared with LPS alone apparently explains the decrease in TXB_2.

In summary, the administration of ET followed by LPS to steers resulted in a decreased inflammatory response compared with administration of LPS alone as indicated by decreased circulating amounts of TNF-α, TXB_2, and Hp. The ET administration resulted in a rapid elevation in circulating cortisol that may have attenuated the LPS-induced TNF-α response. A decrease in the TNF-α response may be a control point such that TXB_2 and Hp are also attenuated. Acute administration of ET alone failed to elicit inflammatory mediators.

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